

been made solely for the purpose of clarifying what subject matter is being claimed, not to avoid the art cited by the Examiner.

Based on the above amendments and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Applicants note the Examiner's objection to the declaration, but respectfully request that this objection be held in abeyance until such time that claims are allowed. *See* 37 C.F.R. § 1.111(b) (1998).

Rejections under 35 U.S.C. § 112

The Examiner has rejected claims 1-20 under 35 U.S.C. § 112, first paragraph, on the grounds that the application allegedly does not enable the full scope of the claims. Applicants respectfully traverse this ground for rejection. The initial burden of establishing a reasonable basis to question the ability of the specification to enable the invention rests with the Examiner. *See* Manual of Patent Examining Procedure (MPEP) § 2164.04 (7th ed. July, 1998); *see also In re Marzocchi*, 169 U.S.P.Q. 367, 369 (C.C.P.A. 1971). The standard for determining whether a claimed invention is enabled is whether one reasonably skilled in the art could make or use the invention, relying on information disclosed in the specification coupled with the information known in the art, without undue experimentation. *See In re Wands*, 8 U.S.P.Q.2d 1400, 1404 (Fed. Cir. 1988); *see also* MPEP § 2164.01 (7th ed. July, 1998).

The Examiner has raised several arguments in support of the enablement rejection. First, the Examiner contends that the specification fails to provide adequate guidance as to the level of expression and of specific activity of inducible macrophage nitric oxide synthase

(iNOS), constitutive neuronal nitric oxide synthase (nNOS), and of deletion/addition mutants of constitutive endothelial nitric oxide synthase (ceNOS), nNOS or iNOS, necessary to induce pulmonary vasodilation and decrease pulmonary arterial pressure, because one of ordinary skill in the art would not expect all isoforms to have the same specific activity in generating NO as ceNOS. To substantiate the allegation that the specification does not enable the use of all isoforms and mutants of NOS in the invention, the Examiner notes that the enzymatic activity of iNOS is calcium independent as compared to ceNOS and nNOS, and that nNOS is significantly different in size and homology from ceNOS.

The specification provides guidance, *inter alia*, in examples 1 and 3, as to the level of expression of a NOS required to produce the desired effect on pulmonary hypertension, and the dosage given to produce that level of expression for ceNOS. With regard to the specific activity of various NOS isoforms required to reduce pulmonary hypertension, the specific activities for various NOS isoforms for several species were known in the art by 1992, *see, e.g.,* Stuehr, D.J. and Griffith, O.W., "Mammalian Nitric Oxide Synthases" in: *Advances in Enzymology and Related Areas of Molecular Biology*, Meister, A., ed., John Wiley & Sons, New York, N. Y., pub., Vol. 65, pp. 287-347, at 295, 296 and 299 (1992)(Attached as Exhibit A), and the specification provides guidance with regard to the activity required to produce the desired affect. The specification discloses, *inter alia*, in Examples 6 and 7 the NOS specific activity required to produce sufficient NO to result in pulmonary vasodilation and treat pulmonary hypertension. Example 6 describes the measurement of ceNOS enzymatic activity found in lung tissue transduced with ceNOS, and in control lung tissue, by assaying for the conversion of L-arginine to L-citrulline. Example 7 describes the measurement of intrapulmonary guanosine 3',5'-cyclic monophosphate (cGMP) levels in

control lungs and in lungs transduced with ceNOS. The specific activity found for ceNOS transduced lungs, defined in terms of L-arginine to L-citrulline conversion or cGMP levels, was sufficient to result in NO production, selective vasodilation of pulmonary vessels, and reduction of pulmonary hypertension as is described in Example 8. All isoforms of NOS catalyze the conversion of L-arginine to L-citrulline with the production of NO, and NO in turn activates soluble guanylate cyclase, which increases intracellular cGMP. (Specification at 3, lines 12-13). Specific activity is measured and described in the specification in terms of the ability to convert L-arginine to L-citrulline, and to produce cGMP, which are activities common to all isoforms of NOS. Thus, the specification discloses for all NOS isoforms, the specific activity required to result in the desired vasodilation, and a method of measuring the specific activity in NOS transduced lung tissue. Moreover, Applicants have now found that when aerosolized adenoviral vector was used to transfer an iNOS gene to rats, iNOS was found to have a higher specific activity, resulting in higher levels of exhaled NO, than eNOS. These results provide evidence that other NOS isoforms may result in a specific activity at least as high as the specific activity of eNOS that resulted in pulmonary vasodilation. In addition, these results indicate that the differences in homology and calcium dependence did not affect the ability of a transferred iNOS to produce sufficient NO.

With regard to the use of NOS addition or deletion mutants in the invention, the specification provides, *inter alia*, at page 12, line 19, to page 14, line 7, ample guidance for the selection of appropriate amino acid substitutions, deletions, or additions that would result in a NOS mutant with the same activity as the wild type for that isoform. In addition, at page 11, lines 19-23, the specification discloses regions of shared homology between ceNOS and nNOS. This disclosure provides guidance to those of ordinary skill in the art as to where

mutations would be most likely to disrupt activity (and would therefore be disfavored), because mutations in more highly conserved sites are more likely to disrupt activity than would mutations in less conserved regions. Moreover, Huang, P.L. and Fishman, which is attached as Exhibit B, relates to the structure-function relationships of the iNOS, nNOS and ceNOS genes. Huang, P.L. and Fishman, M.C., *J. Mol. Med.* 74:415-421 (1996). Thus, relying on the teachings in the specification, in conjunction with other information known in the art, one of ordinary skill in the art could perform targeted mutation of various NOS genes, without affecting the activity of the encoded enzyme. Therefore, at the time of filing, one of ordinary skill in the art could have relied on the guidance provided by the specification, and on the knowledge in the art, to select an appropriate NOS isoform or mutant that would have the NO producing activity necessary to result in pulmonary vasodilation, and reduction of pulmonary hypertension.

The Examiner's second argument in support of the enablement rejection is that due to the alleged unpredictability of gene therapy, and an alleged lack of guidance in the specification regarding routes of administration other than aerosol delivery, the specification does not enable all vector/promoter combinations and all routes of delivery for a nucleic acid encoding a NOS. Where, as here, the Examiner is concerned about the breadth of a generic term, the recitation of the term must be taken as an assertion that all of the species included within the generic term would, as a class, be operative to produce the asserted affect. "The only relevant concern of the Patent Office under these circumstances should be over the truth of any such assertion. The first paragraph of § 112 requires nothing more than objective enablement. How such a teaching is set forth, either by the use of illustrative examples or by broad terminology is of no importance." *In re Marzocchi*, 169 U.S.P.Q. 367, 369 (C.C.P.A.

1971). The burden rests upon the Examiner to show why the teachings, examples and broad recitations in the specification do not enable the claims. *See In re Brana*, 34 U.S.P.Q.2d 1436, 1441 (Fed. Cir. 1995); *see also* MPEP § 2164.04.

A broad allegation that the art of gene therapy is unpredictable, and a conclusory statement that the specification does not provide sufficient guidance with respect to the use of vector-promoter combinations and routes of delivery, is insufficient to meet the burden on Examiner. The Examiner has failed to provide specific reasons why the specification allegedly does not enable the use of vectors or delivery systems other than those disclosed in the working examples. The only explanation provided by the Examiner is a quotation of several statements from Verma, that "the lack of efficient delivery systems, lack of sustained expression, and host immune reactions - remain formidable challenges" in gene therapy, that delivery is the "Achilles heel" of gene therapy, that retroviral vectors cannot "infect non-dividing cells, such as those that make up brain, lung, and liver tissue," and that the choice of an appropriate enhancer-promoter combination is important and that the "search for such combinations is a case of trial and error for a given type of cell." Verma, I.M. and Somia, N., *Nature* 389: 239- 242 (1997).

While these statements were made in Verma, when taken out of context they create a bleaker picture of gene therapy than was probably intended by the authors. For example, the quote from Verma that "retroviral vectors cannot infect non-dividing cells" fails to reflect that Verma discloses that *ex vivo* gene delivery, a form of gene transfer that is disclosed in the specification at page 16, lines 16-17, may be used to overcome this limitation of retroviral

vectors. *Id.* at 240.¹ In addition, Verma discloses that the use of a constitutive promoter in a retroviral vector, transferred *ex vivo*, resulted in prolonged expression of the transgene in muscle tissue, for more than two years (the life of the animal). *Id.* Although Verma focuses on areas of gene therapy that still need development, the article also highlights the level sophistication in gene therapy that existed by the mid-1990's (over 200 clinical gene therapy trials were underway), and concludes that "in the not too distant future, gene therapy will become as routine a practice as heart transplants are today." *Id.* at 242. Thus, the Examiner's reliance on Verma also fails to show why the teachings, examples, and broad recitations in the specification do not enable the use of promoter-vector combinations or routes of delivery other than those disclosed in the examples.

The Examiner's third argument in support of the enablement rejection is that the specification does not enable the administration of a pharmaceutical composition comprising a nucleic acid encoding a NOS, and an immunosuppressive agent or phosphodiesterase inhibitor because allegedly (1) cyclosporin A, one immunosuppressor disclosed in the application, induces hypertension in animals and humans, and may impair the NO generating abilities of NOS, therefore, one of ordinary skill in the art would not have considered a composition containing cyclosporin A and a nucleic acid encoding a NOS to have any pharmaceutical

¹ *Ex vivo* gene delivery does not offer the only solution for delivery of retroviral vectors to lung tissue. For example, an adenovirus might be used to transiently express a retrovirus receptor in cells not usually susceptible to the retrovirus, thereby mediating the uptake of the retrovirus by these cells. This has been done in several human cell lines using adenovirus expressing the ecotropic murine leukemia virus to render the cells susceptible to ecotropic retroviral infection. Scott-Taylor, T.H. *et al.*, *Gene Ther.* 5:621-29 (1998) (Attached as Exhibit C). In addition, retroviral vectors have been successfully used to transduce rabbit tracheal cells *in vivo*, although transduction efficiency was low and occurred only in wounded trachea. Halbert, C.L. *et al.*, *Hum. Gene Ther.* 7:1871-81 (1996) (Attached as Exhibit D).

value; and (2) the specification does not provide sufficient guidance as to dosage or route of delivery for a pharmaceutical composition that combines immunosuppressive agents, or phosphodiesterase inhibitors, with a nucleic acid encoding a NOS.

Roullet *et al.*, on which the Examiner relies for the proposition that cyclosporin A (CysA), induces hypertension, and might impair the NO generating abilities of NOS, reports that CysA-induced hypertension is frequent in solid organ transplantation and autoimmune disease. Roullet, J.-B., *et al.*, *J. Clin. Invest.* 93: 2244-2250, at 2244 (1993). However, this article also suggests that it has not been proven that this hypertension is necessarily due to the effects of CysA. For example, the article states that "in many studies in which altered vascular reactivity was demonstrated, there was no documented elevation of blood pressure, thereby bringing into question the relevance of the observed vascular changes to hypertension," *Id.* at 2244, and that although systemic hypertension has been reproduced in animals given CysA, the data are inconsistent, *id.* at 2246. Moreover, the discussion in Roullet *et al.* about the effect of CysA on the NO generating system is inconclusive. On this subject, Roullet *et al.* states that "the molecular mechanism of action of the drug has not been explored in our study and remains to be determined." Thus, this article cannot be relied upon to sustain the proposition that one of ordinary skill in the art would not have considered a composition containing both CysA and a nucleic acid encoding a NOS to be of pharmaceutical value.

The Examiner has also contended that the application does not provide sufficient guidance as to the dosages or routes of delivery for the administration of a pharmaceutical composition containing a nucleic acid encoding a NOS, and an immunosuppressive agent or a phosphodiesterase inhibitor. However, phosphodiesterase inhibitors and immunosuppressive agents were well known in the art at the time of filing, as evidenced by Cohen *et al.*, *J. Clin.*

Invest. 97:172-179 (1996), which was cited in the specification at page 17 line 5, and incorporated into the specification by reference, as well as by Roullet, J.-B. *et al.*, *J. Clin. Invest. 93*: 2244-2250 (1994), cited by the Examiner. Therefore, determining dosages and routes of delivery for these agents would be routine and would not require undue experimentation. *See Ex parte Rubin*, 5 USPQ2d 1461, 1462 (Bd. Pat. App. & Int. 1987) (new method of using old compounds found enabled, where modes of administering compounds well known in art); *see also* MPEP § 2164.01(c).

The fourth and final aspect of the enablement rejection is that the specification allegedly does not enable the treatment of any and all forms of primary or secondary pulmonary hypertension in all mammals. In support of this allegation, the Examiner first states that a common characteristic of most forms of primary and secondary pulmonary hypertension is neointimal formation and physical narrowing of the artery as a result of smooth muscle cell migration and proliferation, implying that to enable the treatment of pulmonary hypertension, the specification must provide evidence that the administration of NOS can affect these changes in the artery. The Examiner contends that the specification does not provide this evidence because, according to Heath, D., *Eur Respir. Rev.* 3:555-558 (1993), (1) the rat, which is used in the working examples, is a poor model for pulmonary hypertension as the form of hypertensive pulmonary vascular disease induced in the rat is due to muscular evaginations rather than migration of vascular smooth muscle cells found in human hypertensive pulmonary disease; (2) although vasodilators may reverse the vasoconstrictive component of pulmonary hypertension, their effect on intimal changes is unknown; and (3) pulmonary vasodilators are unlikely to be effective once migration of vascular smooth muscle cells has occurred.

Assuming, *arguendo*, that the Examiner is correct in requiring evidence that NOS affects the changes in the artery to enable treatment of pulmonary hypertension using NOS, the Examiner has mischaracterized the statements made in Heath. The Heath article provides a general discussion of pulmonary hypertension, but does not address the ability of NO or NO synthases to affect pulmonary hypertension, vascular smooth muscle cell migration or proliferation, or neointimal formation. The article does state that "[p]ulmonary vasodilators are unlikely to be effective once migration of vascular smooth muscle cells has occurred," *id.* at 555, abstract, and that "[w]hile [pulmonary vasodilators] are likely to reverse any vasoconstrictive component, it is difficult to conceive what effect they might have on structural changes in the intima brought about by limited or free migration of vascular smooth muscle cells" *Id.* at 557-558. However, immediately after the second statement, the article indicates that one "vasodilator," ligustrazine, appears to act by attenuating the growth of new muscle in pulmonary arterioles, and suggests therefore, that a new and broader term is needed for those vasodilators that also ameliorate the remodeling involved in pulmonary hypertension. *Id.* at 558. The statements in Heath about vasodilators, therefore, are not intended to describe every compound that can be described as a vasodilator, and shed no light on the effects of NO on pulmonary hypertension. Thus, the Examiner cannot rely on these general statements in Heath to meet the burden of providing a reasonable basis to question the ability of the specification to enable the invention.

Moreover, contrary to the statement in the abstract of Heath, that in the rat hypoxia induces vasoconstriction with muscular evaginations, but not migration of smooth muscle cells, Roberts *et al.*, which is attached as Exhibit E, states that in the rat during chronic hypoxia pulmonary vessel wall smooth muscle cells do undergo hypertrophy and/or

hyperplasia and proliferate in the walls of peripheral arteries. Roberts, J.D. *et al.*, *Circulation Res.* 76:215-222, at 215 (1995). Roberts *et al.*, also states that the pulmonary vascular remodeling that occurs in humans residing at high altitudes can be reproduced in newborn and infant rats breathing air with reduced oxygen tension for several weeks. *Id.* Thus, Roberts *et al.*, provides support for the use of the rat as a model for pulmonary hypertension in humans. In addition, Roberts *et al.* reports that breathing NO for two weeks decreased the hypoxic pulmonary vascular structural changes that normally accompany chronic hypoxia in newborn rats. Thus, Roberts *et al.* provides evidence that NO may affect the arterial changes found in pulmonary hypertension, and could act on pulmonary hypertension through mechanisms other than vasodilation. Furthermore, Applicants have now confirmed the inhibitory effect of NO on the structural changes in the pulmonary vasculature that accompany chronic hypoxic pulmonary hypertension. These experiments indicate that transfer of a NOS gene suppressed the muscular hypertrophy that accompanies hypoxia, in addition to affecting vasodilation. Thus, the allegation that the specification does not enable the treatment of all forms of pulmonary hypertension with NOS gene therapy, because NOS does not appear to affect vascular remodeling, is incorrect.

The Examiner has also alleged that with regard to the prophylactic use of NOS to prevent the development of pulmonary hypertension, the specification does not provide guidance for the administration of a nucleic acid encoding NOS to patients with a cardiac septal defect or thrombotic disease for the complete prevention of the disease. The initial burden is on the Examiner to establish a reasonable basis to question the ability of the specification to enable the invention. MPEP § 2164.04. This general statement does not provide any reason for questioning the ability of the specification to enable the use of a nucleic

acid encoding a NOS to treat of pulmonary hypertension prophylactically. The Examiner's statement that clinical efficacy has not yet been definitively demonstrated for any gene therapy protocol may have been intended to provide support for the allegation that the specification does not enable the use of a NOS to treat pulmonary hypertension prophylactically. However, even assuming this statement to be true, Applicants are not required to provide evidence of clinical efficacy to meet the patentability requirements for the administration of an agent for the treatment of a disease. *See In re Brana*, 34 U.S.P.Q.2d 1436, 1442 (Fed. Cir. 1995). Thus, this argument also fails to meet the Examiner's burden. Applicants submit that the enablement rejection is improper and respectfully request that it be withdrawn.

The Examiner has also rejected claims 3, 9 and 17 under 35 U.S.C. § 112, second paragraph, for alleged indefiniteness. Applicants respectfully traverse this ground for rejection. Specifically, the Examiner rejected claims 3 and 9 for a lack of antecedent basis. The amendment above corrects errors in these claims thereby providing an antecedent basis for all elements of these claims. In addition, the Examiner has found claim 17 indefinite due to an allegedly improper use of a "means" clause. Even if this rejection were proper, the amendment above renders this rejection moot because claim 17 no longer recites a "means clause." Therefore, Applicants submit that the rejection of claims 3, 9 and 17 for indefiniteness is now moot and respectfully request that it be withdrawn.

Rejections under 35 U.S.C. § 103

The Examiner has rejected claim 17 under 35 U.S.C. § 103(a) as allegedly obvious over Chen, A.F.Y. *et al.*, *FASEB J.* 10:A303, Abstract No. 1743 (March 8, 1996) in view of Rosenfeld, M.A. *et al.*, *Cell* 68:143-155 (January 1992). Applicants do not admit that these

articles qualify as prior art, and reserve the right to establish that they are not.² However, Applicants traverse this rejection based on the content of the cited articles.

Claim 17, as amended, claims a pharmaceutical composition comprising a nucleic acid encoding a nitric oxide synthase operably linked to an expression control element for selective expression of said nucleic acid in pulmonary tissue, and a pharmaceutically acceptable carrier vehicle. To establish a *prima facie* case of obviousness, the articles cited by the Examiner should both (1) provide a suggestion or motivation to those of ordinary skill in the art to make the claimed composition; and (2) reveal that one of ordinary skill would have a reasonable expectation of success in doing so. *See In re Vaeck*, 20 U.S.P.Q.2d 1438, 1442 (Fed. Cir. 1991); *see also* MPEP § 706.02(j) (7th Ed. July, 1998). In addition, the reference, or references must teach all of the claim limitations. MPEP § 706.02(j). The rejection does not meet even the first requirement.

The Examiner cites Chen *et al.* for its teaching of an adenoviral vector encoding bovine ceNOS linked to a CMV promoter, and the transfer of this vector *ex vivo* to basilar artery rings resulting in expression of the transgene. Chen *et al.*, does not teach the selective expression of the gene carried by the vector, *in vivo* or otherwise, in pulmonary tissue. Thus, Chen *et al.*, provides no motivation to provide a pharmaceutical composition containing the nucleic acid of the invention having an expression control element specific for pulmonary tissue, and a pharmaceutically acceptable carrier vehicle.

Rosenfeld *et al.*, does not provide this motivation. The Examiner relies on Rosenfeld *et al.*, for its teaching that adenovirus is tropic for respiratory epithelium, and that an

² Applicants note that aspects of this invention are disclosed in Janssens, S.P. *et al.*, *J. Clin. Invest.* 98:317-324 (1996) (not prior art), which was submitted for publication on December 27, 1995, the date the manuscript on which the present application was based.

adenoviral vector carrying the cystic fibrosis transmembrane conductance regulator (CFTR) gene was able to transduce pulmonary tissue resulting in expression of the transgene. The Examiner alleges that this teaching provides the motivation to use an adenoviral vector to transduce lung tissue *in vivo*. Even assuming, *arguendo*, that this is true, the fact that a particular viral vector can be used transduce lung tissue does not provide the motivation to provide that vector carrying a different gene, NOS, for the transduction of lung. The ability to use a particular viral vector to transduce a particular type of tissue does not provide the motivation to prepare that vector carrying any gene to transduce that type of tissue.

The cited articles fail to provide a motivation to prepare a vector, targeted to lung tissue and *carrying a NOS gene*, in a pharmaceutical composition. Thus, the Examiner has failed to make a *prima facie* showing of obviousness. For this reason, Applicants respectfully request that the rejection of claim 17 under 35 U.S.C. § 103(a) be withdrawn.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

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